<110> Hildinger, Markus

<120> Decreasing gene expression in a mammalian subject in vivo via
AAV-mediated RNAi expression cassette transfer

<130> 2

<140> US 10/604,340
<141> 2003-07-13

<160> 12

<170> PatentIn version 3.2

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plasmid backbone, with AAV2 internal terminal repeats that flank
expression cassette; referred to as AAV2/2 CMV luciferase

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Phe Thr Asp Ala His Ile Glu Val Asp Ile Thr Tyr Ala Glu Tyr Phe
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Glu Met Ser Val Arg Leu Ala Glu Ala Met Lys Arg Tyr Gly Leu Asn
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Gln Pro Thr Val Val Phe Val Ser Lys Lys Gly Leu Gln Lys Ile Leu
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aac gtg caa aaa aag ctc cca atc atc caa aaa att att atc atg gat     1686
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Asp Thr Ala Ile Leu Ser Val Val Pro Phe His His Gly Phe Gly Met
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att caa agt gcg ctg ctg gtg cca acc cta ttc tcc ttc ttc gcc aaa        2118
Ile Gln Ser Ala Leu Leu Val Pro Thr Leu Phe Ser Phe Phe Ala Lys
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agc act ctg att gac aaa tac gat tta tct aat tta cac gaa att gct        2166
Ser Thr Leu Ile Asp Lys Tyr Asp Leu Ser Asn Leu His Glu Ile Ala
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tct ggt ggc gct ccc ctc tct aag gaa gtc ggg gaa gcg gtt gcc aag        2214
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Arg Phe His Leu Pro Gly Ile Arg Gln Gly Tyr Gly Leu Thr Glu Thr
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Thr Ser Ala Ile Leu Ile Thr Pro Glu Gly Asp Asp Lys Pro Gly Ala
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Val Gly Lys Val Val Pro Phe Phe Glu Ala Lys Val Val Asp Leu Asp
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gcc ttg att gac aag gat gga tgg cta cat tct gga gac ata gct tac        2502
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Val Thr Thr Ala Lys Lys Leu Arg Gly Gly Val Val Phe Val Asp Glu
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Val Asp Ile Thr Tyr Ala Glu Tyr Phe Glu Met Ser Val Arg Leu Ala
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Glu Ala Met Lys Arg Tyr Gly Leu Asn Thr Asn His Arg Ile Val Val
65                  70                  75                  80


Cys Ser Glu Asn Ser Leu Gln Phe Phe Met Pro Val Leu Gly Ala Leu
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Phe Ile Gly Val Ala Val Ala Pro Ala Asn Asp Ile Tyr Asn Glu Arg
            100                 105                 110


Glu Leu Leu Asn Ser Met Gly Ile Ser Gln Pro Thr Val Val Phe Val
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Ser Lys Lys Gly Leu Gln Lys Ile Leu Asn Val Gln Lys Lys Leu Pro
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Asn Glu Tyr Asp Phe Val Pro Glu Ser Phe Asp Arg Asp Lys Thr Ile
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Pro Ile Phe Gly Asn Gln Ile Ile Pro Asp Thr Ala Ile Leu Ser Val
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Val Pro Phe His His Gly Phe Gly Met Phe Thr Thr Leu Gly Tyr Leu
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Ile Cys Gly Phe Arg Val Val Leu Met Tyr Arg Phe Glu Glu Glu Leu
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Glu Ile Val Asp Tyr Val Ala Ser Gln Val Thr Thr Ala Lys Lys Leu
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Gly Pro Ala Pro Phe Tyr Pro Leu Glu Asp Gly Thr Ala Gly Glu Gln
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Leu His Lys Ala Met Lys Arg Tyr Ala Leu Val Pro Gly Thr Ile Ala
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ttt aca gat gca cat atc gag gtg gac atc act tac gct gag tac ttc    1398
Phe Thr Asp Ala His Ile Glu Val Asp Ile Thr Tyr Ala Glu Tyr Phe
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aac gtg caa aaa aag ctc cca atc atc caa aaa att att atc atg gat    1686
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Ser His Leu Pro Pro Gly Phe Asn Glu Tyr Asp Phe Val Pro Glu Ser
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Phe Asp Arg Asp Lys Thr Ile Ala Leu Ile Met Asn Ser Ser Gly Ser
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act ggt ctg cct aaa ggt gtc gct ctg cct cat aga act gcc tgc gtg    1878
Thr Gly Leu Pro Lys Gly Val Ala Leu Pro His Arg Thr Ala Cys Val
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aga ttc tcg cat gcc aga gat cct att ttt ggc aat caa atc att ccg    1926
Arg Phe Ser His Ala Arg Asp Pro Ile Phe Gly Asn Gln Ile Ile Pro
            220                 225                 230

gat act gcg att tta agt gtt gtt cca ttc cat cac ggt ttt gga atg    1974
Asp Thr Ala Ile Leu Ser Val Val Pro Phe His His Gly Phe Gly Met
    235                 240                 245

ttt act aca ctc gga tat ttg ata tgt gga ttt cga gtc gtc tta atg    2022
Phe Thr Thr Leu Gly Tyr Leu Ile Cys Gly Phe Arg Val Val Leu Met
250                 255                 260                 265

tat aga ttt gaa gaa gag ctg ttt ctg agg agc ctt cag gat tac aag    2070
Tyr Arg Phe Glu Glu Glu Leu Phe Leu Arg Ser Leu Gln Asp Tyr Lys
                270                 275                 280

att caa agt gcg ctg ctg gtg cca acc cta ttc tcc ttc ttc gcc aaa    2118
Ile Gln Ser Ala Leu Leu Val Pro Thr Leu Phe Ser Phe Phe Ala Lys
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agc act ctg att gac aaa tac gat tta tct aat tta cac gaa att gct    2166
Ser Thr Leu Ile Asp Lys Tyr Asp Leu Ser Asn Leu His Glu Ile Ala
        300                 305                 310

tct ggt ggc gct ccc ctc tct aag gaa gtc ggg gaa gcg gtt gcc aag    2214
Ser Gly Gly Ala Pro Leu Ser Lys Glu Val Gly Glu Ala Val Ala Lys
        315                 320                 325

agg ttc cat ctg cca ggt atc agg caa gga tat ggg ctc act gag act    2262
Arg Phe His Leu Pro Gly Ile Arg Gln Gly Tyr Gly Leu Thr Glu Thr
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Val Gly Lys Val Val Pro Phe Phe Glu Ala Lys Val Val Asp Leu Asp
            365                 370                 375

acc ggg aaa acg ctg ggc gtt aat caa aga ggc gaa ctg tgt gtg aga   2406
Thr Gly Lys Thr Leu Gly Val Asn Gln Arg Gly Glu Leu Cys Val Arg
            380                 385                 390

ggt cct atg att atg tcc ggt tat gta aac aat ccg gaa gcg acc aac   2454
Gly Pro Met Ile Met Ser Gly Tyr Val Asn Asn Pro Glu Ala Thr Asn
            395                 400                 405

gcc ttg att gac aag gat gga tgg cta cat tct gga gac ata gct tac   2502
Ala Leu Ile Asp Lys Asp Gly Trp Leu His Ser Gly Asp Ile Ala Tyr
410                 415                 420                 425

tgg gac gaa gac gaa cac ttc ttc atc gtt gac cgc ctg aag tct ctg   2550
Trp Asp Glu Asp Glu His Phe Phe Ile Val Asp Arg Leu Lys Ser Leu
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att aag tac aaa ggc tat cag gtg gct ccc gct gaa ttg gaa tcc atc   2598
Ile Lys Tyr Lys Gly Tyr Gln Val Ala Pro Ala Glu Leu Glu Ser Ile
            445                 450                 455

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Leu Leu Gln His Pro Asn Ile Phe Asp Ala Gly Val Ala Gly Leu Pro
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gac gat gac gcc ggt gaa ctt ccc gcc gcc gtt gtt gtt ttg gag cac   2694
Asp Asp Asp Ala Gly Glu Leu Pro Ala Ala Val Val Val Leu Glu His
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Gly Lys Thr Met Thr Glu Lys Glu Ile Val Asp Tyr Val Ala Ser Gln
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Val Thr Thr Ala Lys Lys Leu Arg Gly Gly Val Val Phe Val Asp Glu
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gta ccg aaa ggt ctt acc gga aaa ctc gac gca aga aaa atc aga gag   2838
Val Pro Lys Gly Leu Thr Gly Lys Leu Asp Ala Arg Lys Ile Arg Glu
            525                 530                 535

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Ile Leu Ile Lys Ala Lys Lys Gly Gly Lys Ile Ala Val
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Tyr Ala Leu Val Pro Gly Thr Ile Ala Phe Thr Asp Ala His Ile Glu
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Val Asp Ile Thr Tyr Ala Glu Tyr Phe Glu Met Ser Val Arg Leu Ala
        50              55              60

Glu Ala Met Lys Arg Tyr Gly Leu Asn Thr Asn His Arg Ile Val Val
65              70              75              80

Cys Ser Glu Asn Ser Leu Gln Phe Phe Met Pro Val Leu Gly Ala Leu
            85              90              95

Phe Ile Gly Val Ala Val Ala Pro Ala Asn Asp Ile Tyr Asn Glu Arg
            100             105             110

Glu Leu Leu Asn Ser Met Gly Ile Ser Gln Pro Thr Val Val Phe Val
        115             120             125

Ser Lys Lys Gly Leu Gln Lys Ile Leu Asn Val Gln Lys Lys Leu Pro
    130             135             140

Ile Ile Gln Lys Ile Ile Ile Met Asp Ser Lys Thr Asp Tyr Gln Gly
145             150             155             160

Phe Gln Ser Met Tyr Thr Phe Val Thr Ser His Leu Pro Pro Gly Phe
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Asn Glu Tyr Asp Phe Val Pro Glu Ser Phe Asp Arg Asp Lys Thr Ile
            180             185             190

Ala Leu Ile Met Asn Ser Ser Gly Ser Thr Gly Leu Pro Lys Gly Val
        195             200             205

Ala Leu Pro His Arg Thr Ala Cys Val Arg Phe Ser His Ala Arg Asp
        210             215             220

Pro Ile Phe Gly Asn Gln Ile Ile Pro Asp Thr Ala Ile Leu Ser Val
225             230             235             240

Val Pro Phe His His Gly Phe Gly Met Phe Thr Thr Leu Gly Tyr Leu
            245             250             255

Ile Cys Gly Phe Arg Val Val Leu Met Tyr Arg Phe Glu Glu Glu Leu
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Phe Leu Arg Ser Leu Gln Asp Tyr Lys Ile Gln Ser Ala Leu Leu Val
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Pro Thr Leu Phe Ser Phe Phe Ala Lys Ser Thr Leu Ile Asp Lys Tyr
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Asp Leu Ser Asn Leu His Glu Ile Ala Ser Gly Gly Ala Pro Leu Ser
305             310             315             320

Lys Glu Val Gly Glu Ala Val Ala Lys Arg Phe His Leu Pro Gly Ile
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Arg Gln Gly Tyr Gly Leu Thr Glu Thr Thr Ser Ala Ile Leu Ile Thr
            340             345             350

Pro Glu Gly Asp Asp Lys Pro Gly Ala Val Gly Lys Val Val Pro Phe
        355             360             365

Phe Glu Ala Lys Val Val Asp Leu Asp Thr Gly Lys Thr Leu Gly Val
    370             375             380

Asn Gln Arg Gly Glu Leu Cys Val Arg Gly Pro Met Ile Met Ser Gly
385             390             395             400

Tyr Val Asn Asn Pro Glu Ala Thr Asn Ala Leu Ile Asp Lys Asp Gly
            405             410             415

Trp Leu His Ser Gly Asp Ile Ala Tyr Trp Asp Glu Asp Glu His Phe
            420             425             430

Phe Ile Val Asp Arg Leu Lys Ser Leu Ile Lys Tyr Lys Gly Tyr Gln
    435             440             445

Val Ala Pro Ala Glu Leu Glu Ser Ile Leu Leu Gln His Pro Asn Ile
    450             455             460

Phe Asp Ala Gly Val Ala Gly Leu Pro Asp Asp Asp Ala Gly Glu Leu
465             470             475             480

Pro Ala Ala Val Val Val Leu Glu His Gly Lys Thr Met Thr Glu Lys
            485             490             495

Glu Ile Val Asp Tyr Val Ala Ser Gln Val Thr Thr Ala Lys Lys Leu
            500             505             510

Arg Gly Gly Val Val Phe Val Asp Glu Val Pro Lys Gly Leu Thr Gly
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Lys Leu Asp Ala Arg Lys Ile Arg Glu Ile Leu Ile Lys Ala Lys Lys
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Gly Gly Lys Ile Ala Val
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<213>  Artificial ·

<220>
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       plasmid backbone, with AAV2 internal terminal repeats that flank
       expression cassette; referred to as AAV2/5 U6 lucRI-1a

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       expression cassette; referred to as AAV2/5 U6 lucRI-1b

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caaccctatc tcggtctatt cttttgattt ataagggatt tttccgattt cggcctattg      1920

gttaaaaaat gagctgattt aacaaaaatt taacgcgaat tttaacaaaa tattaacgtt      1980

tataatttca ggtggcatct ttcggggaaa tgtgcgcgga acccctattt gtttattttt      2040

ctaaatacat tcaaatatgt atccgctcat gagacaataa ccctgataaa tgcttcaata      2100

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ccttgagagt tttcgccccg aagaacgttt tccaatgatg agcactttta aagttctgct      2340

atgtggcgcg gtattatccc gtattgacgc cgggcaagag caactcggtc gccgcataca      2400

ctattctcag aatgacttgg ttgagtactc accagtcaca gaaaagcatc ttacggatgg      2460

catgacagta agagaattat gcagtgctgc cataaccatg agtgataaca ctgcggccaa      2520

cttacttctg acaacgatcg gaggaccgaa ggagctaacc gcttttttgc acaacatggg      2580

ggatcatgta actcgccttg atcgttggga accggagctg aatgaagcca taccaaacga      2640

cgagcgtgac accacgatgc ctgtagtaat ggtaacaacg ttgcgcaaac tattaactgg      2700

cgaactactt actctagctt cccggcaaca attaatagac tggatggagg cggataaagt      2760

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ccttttttgat aatctcatga ccaaaatccc ttaacgtgag ttttcgttcc actgagcgtc      3120

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ctgcttgcaa acaaaaaaac caccgctacc agcggtggtt tgtttgccgg atcaagagct      3240

accaactctt tttccgaagg taactggctt cagcagagcg cagataccaa atactgtcct      3300

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ctgcggtttt gctcacatgt tctttcctgc gttatcccct gattctgtgg ataaccgtat      3840

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agtgagcgag gaagcggaag                                                  3920


<210> 7
<211> 3923
<212> DNA
<213> Artificial

<220>
<223> sequence for recombinant adeno-associated viral vector, including
      plasmid backbone, with AAV2 internal terminal repeats that flank
      expression cassette; referred to as AAV2/5 U6/U6 lucRIU6-3

<400> 7
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tcactcatta ggcaccccag gctttacact ttatgcttcc ggctcgtatg ttgtgtggaa      180

ttgtgagcgg ataacaattt cacacaggaa acagctatga ccatgattac gccagattta      240

attaaggctg cgcgctcgct cgctcactga ggccgcccgg gcaaagcccg ggcgtcgggc      300

gacctttggt cgcccggcct cagtgagcga gcgagcgcgc agagagggag tggccaactc      360

catcactagg ggttccttgt agttaatgat taacccgcca tgctacttat ctacgtagcc      420

atgctctagg aagatcggaa ttcgcccta agctagcccc cagtggaaag acgcgcaggc      480

aaaacgcacc acgtgacgga gcgtgaccgc gcgccgagcc caaggtcggg caggaagagg      540

gcctatttcc catgattcct tcatatttgc atatacgata caaggctgtt agagagataa      600
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ttagaattaa tttgactgta aacacaaaga tattagtaca aaatacgtga cgtagaaagt    660

aataatttct tgggtagttt gcagttttaa aattatgttt taaaatggac tatcatatgc    720

ttaccgtaac ttgaaagtat ttcgatttct tggctttata tatcttgtgg aaaggacgaa    780

acacctttt cttacgctga gtacttcgat ttttggtgtt cgtcctttc cacaagatat    840

ataaagccaa gaaatcgaaa tactttcaag ttacggtaag catatgatag tccattttaa    900

aacataattt taaaactgca aactacccaa gaaattatta ctttctacgt cacgtatttt    960

gtactaatat ctttgtgttt acagtcaaat taattctaat tatctctcta acagccttgt   1020

atcgtatatg caaatatgaa ggaatcatgg gaaataggcc ctcttcctgc ccgaccttgg   1080

gctcggcgcg cggtcacgct ccgtcacgtg gtgcgttttg cctgcgcgtc tttccactgg   1140

ggctcgagtt aagggcgaat tcccgattag gatcttccta gagcatggct acgtagataa   1200

gtagcatggc gggttaatca ttaactacaa ggaacccta gtgatggagt tggccactcc   1260

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ggccgtcgtt ttacaacgtc gtgactggga aaaccctggc gttacccaac ttaatcgcct   1440

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actcaaccct atctcggtct attcttttga tttataaggg attttttccga tttcggccta   1920

ttggttaaaa aatgagctga tttaacaaaa atttaacgcg aattttaaca aaatattaac   1980

gtttataatt tcaggtggca tctttcgggg aaatgtgcgc ggaacccta tttgtttatt   2040

tttctaaata cattcaaata tgtatccgct catgagacaa taaccctgat aaatgcttca   2100

ataatattga aaaaggaaga gtatgagtat tcaacatttc cgtgtcgccc ttattccctt   2160

ttttgcggca ttttgccttc ctgttttttgc tcacccagaa acgctggtga agtaaaaga   2220

tgctgaagat cagttgggtg cacgagtggg ttacatcgaa ctggatctca atagtggtaa   2280

gatccttgag agttttcgcc ccgaagaacg ttttccaatg atgagcactt ttaaagttct   2340

gctatgtggc gcggtattat cccgtattga cgccgggcaa gagcaactcg gtcgccgcat   2400

acactattct cagaatgact tggttgagta ctcaccagtc acagaaaagc atcttacgga   2460
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tggcatgaca gtaagagaat tatgcagtgc tgccataacc atgagtgata acactgcggc      2520

caacttactt ctgacaacga tcggaggacc gaaggagcta accgcttttt tgcacaacat      2580

gggggatcat gtaactcgcc ttgatcgttg ggaaccggag ctgaatgaag ccataccaaa      2640

cgacgagcgt gacaccacga tgcctgtagt aatggtaaca acgttgcgca aactattaac      2700

tggcgaacta cttactctag cttcccggca acaattaata gactggatgg aggcggataa      2760

agttgcagga ccacttctgc gctcggccct tccggctggc tggtttattg ctgataaatc      2820

tggagccggt gagcgtgggt ctcgcggtat cattgcagca ctggggccag atggtaagcc      2880

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acagatcgct gagataggtg cctcactgat taagcattgg taactgtcag accaagttta      3000

ctcatatata ctttagattg atttaaaact tcatttttaa tttaaaagga tctaggtgaa      3060

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gtcagacccc gtagaaaaga tcaaaggatc ttcttgagat cctttttttc tgcgcgtaat      3180

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gctaccaact ctttttccga aggtaactgg cttcagcaga gcgcagatac caaatactgt      3300

ccttctagtg tagccgtagt taggccacca cttcaagaac tctgtagcac cgcctacata      3360

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ttatagtcct gtcgggtttc gccacctctg acttgagcgt cgatttttgt gatgctcgtc      3720

agggggggcgg agcctatgga aaaacgccag caacgcggcc ttttttacggt tcctggcctt      3780

ttgctgcggt tttgctcaca tgttctttcc tgcgttatcc cctgattctg tggataaccg      3840

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<210> 8
<211> 3589
<212> DNA
<213> Artificial

<220>
<223> sequence for recombinant adeno-associated viral vector, including
      plasmid backbone, with AAV2 internal terminal repeats that flank
      expression cassette; referred to as AAV2/5 U6 lucRI-4(sense)

<400> 8
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tcactcatta ggcacccag gctttacact ttatgcttcc ggctcgtatg ttgtgtggaa  180

ttgtgagcgg ataacaattt cacacaggaa acagctatga ccatgattac gccagattta  240

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catcactagg ggttccttgt agttaatgat taacccgcca tgctacttat ctacgtagcc  420

atgctctagg aagatcggaa ttcgccctta agctagcccc cagtggaaag acgcgcaggc  480

aaaacgcacc acgtgacgga gcgtgaccgc gcgccgagcc caaggtcggg caggaagagg  540

gcctatttcc catgattcct tcatatttgc atatacgata caaggctgtt agagagataa  600

ttagaattaa tttgactgta aacacaaaga tattagtaca aaatacgtga cgtagaaagt  660

aataatttct tgggtagttt gcagttttaa aattatgttt taaaatggac tatcatatgc  720

ttaccgtaac ttgaaagtat ttcgatttct tggctttata tatcttgtgg aaaggacgaa  780

acacccttac gctgagtact tcgattttct cgagttaagg gcgaattccc gattaggatc  840

ttcctagagc atggctacgt agataagtag catggcgggt taatcattaa ctacaaggaa  900

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cgaccaaagg tcgcccgacg cccgggcttt gcccggggcgg cctcagtgag cgagcgagcg  1020

cgcagcctta attaacctaa ttcactggcc gtcgttttac aacgtcgtga ctgggaaaac  1080

cctggcgtta cccaacttaa tcgccttgca gcacatcccc ctttcgccag ctggcgtaat  1140

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ccagaaacgc tggtgaaagt aaaagatgct gaagatcagt tgggtgcacg agtgggttac  1920
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gggcaagagc aactcggtcg ccgcatacac tattctcaga atgacttggt tgagtactca   2100

ccagtcacag aaaagcatct tacggatggc atgacagtaa gagaattatg cagtgctgcc   2160

ataaccatga gtgataacac tgcggccaac ttacttctga caacgatcgg aggaccgaag   2220

gagctaaccg cttttttgca caacatgggg gatcatgtaa ctcgccttga tcgttgggaa   2280

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gcggtggttt gtttgccgga tcaagagcta ccaactcttt ttccgaaggt aactggcttc   2940

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aagaactctg tagcaccgcc tacatacctc gctctgctaa tcctgttacc agtggctgct   3060

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ttatcccctg attctgtgga taaccgtatt accgcctttg agtgagctga taccgctcgc   3540

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<210>  9
<211>  3589
<212>  DNA
<213>  Artificial

<220>
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<223> sequence for recombinant adeno-associated viral vector, including
      plasmid backbone, with AAV2 internal terminal repeats that flank
      expression cassette; referred to as AAV2/5 U6 lucRI-4(antisense)

<400> 9

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      plasmid backbone, with AAV2 internal terminal repeats that flank
      expression cassette; referred to as AAV2/2 U6 eGFPRI-1a

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<213> Artificial

<220>
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       plasmid backbone, with AAV2 internal terminal repeats that flank
       expression cassette; referred to as AAV2/5 poll lucRI

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       expression cassette; referred to as AAV2/5 U6/U6 lucRI-2

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